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U. S. DEPARTMENT OF AGRICULTURE.

DIVISION OF VEGETABLE PHYSIOLOGY AND PATHOLOGY.

COPPER SULPHATE AND GERMINATION.

TREATMENT OF SEED WITH COPPER SULPHATE
TO PREVENT THE ATTACKS OF FUNGI.

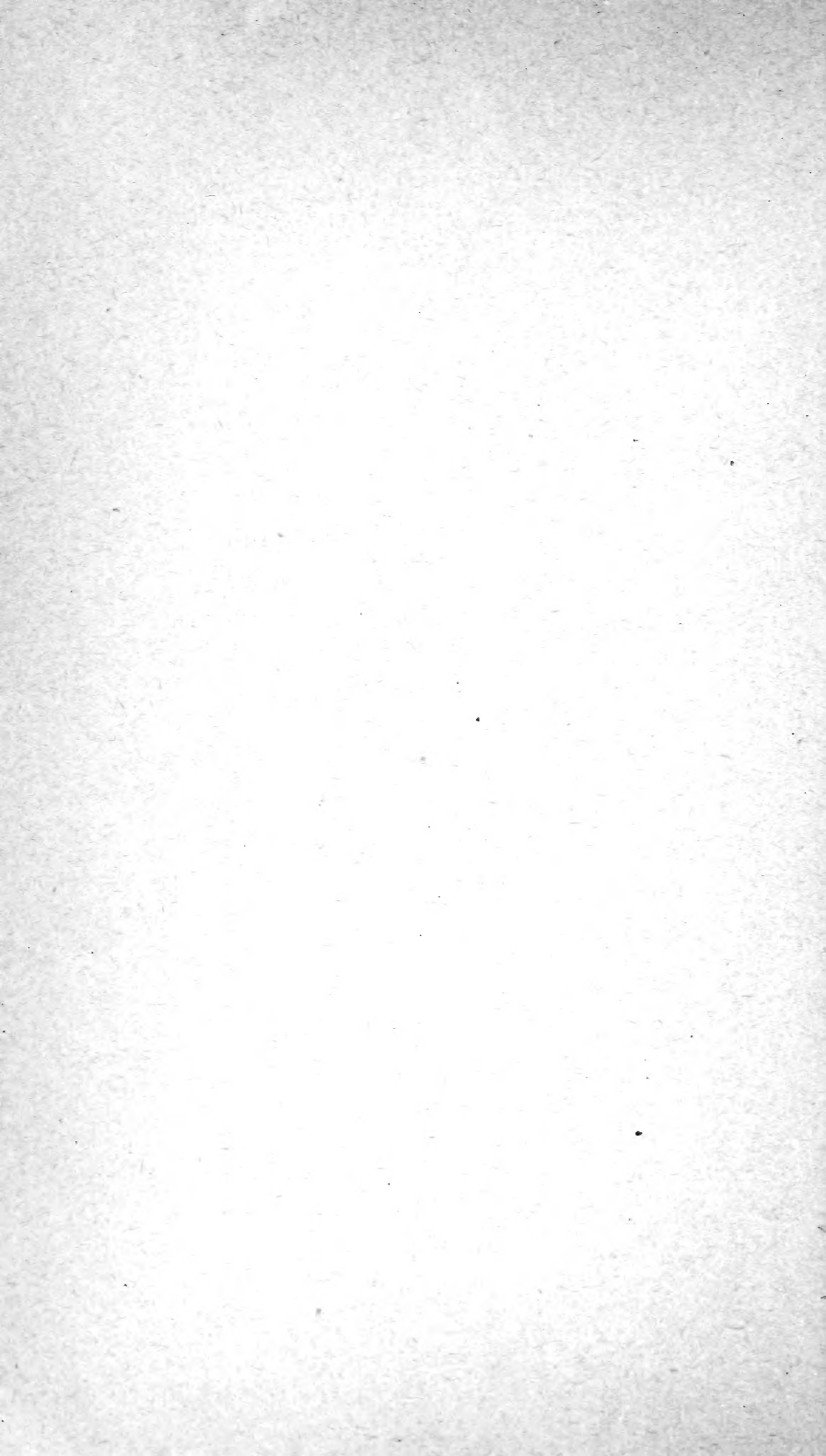
BY

WALTER H. EVANS, Ph. D.



WASHINGTON:
GOVERNMENT PRINTING OFFICE.
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LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
DIVISION OF VEGETABLE PHYSIOLOGY AND PATHOLOGY,
Washington, D. C., June 8, 1896.

SIR: I have the honor to transmit herewith a paper on the treatment of seed with copper sulphate to prevent the attacks of fungi, by Dr. Walter H. Evans, of the Office of Experiment Stations. Although the paper was prepared independent of this division, I would recommend, in view of its nature, that it be published as a bulletin of our regular series.

Respectfully,

B. T. GALLOWAY,
Chief of Division.

HON. J. STERLING MORTON,
Secretary of Agriculture.

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TREATMENT OF SEED WITH COPPER SULPHATE TO PREVENT THE ATTACKS OF FUNGI.

INTRODUCTION.

The object of treating seed with some chemical before planting is fourfold: (1) To hasten germination, (2) to furnish nourishment for the young plant, (3) to protect the seed from insect and other animal pests, and (4) to prevent the attacks of fungi. Investigations have been conducted with greater or less success in all these lines, but it is only with the last that this paper is concerned.

To prevent the attacks of fungi many kinds of seed treatment have been tried. These treatments may be classified under two heads—treatment with chemicals and treatment with heat. It has long been known that where the spores of a fungus are present on seed when sown the fungus is able to infect many plants. The spores of many smuts germinate simultaneously with the seed, and finding entrance into the delicate tissues of the young plant, the fungus is safe from all external treatments, and its presence remains unsuspected until about the time of the maturity of the plant, when it reappears to infect the seed of the host. In this way the life cycles of many parasitic fungi are passed; hence, if the development of the plant could in some way be hastened or the growth of the spores of the fungus retarded until the young plants have made even a small amount of growth, they would be practically secure from infection, or if the spores adhering to the seed could be destroyed without injuring the seed itself all danger from parasites would be removed.

Nobbe,¹ quoting Kühn, states that the spores of grain smuts will retain their vitality for a year or more, and that if these spores could be prevented from germinating, or if their germination could be delayed for a short time, much if not all the injury done to our grain crops would be avoided.

Kühn² claims that the time during which a fungus can infect a plant is limited to a very brief period in the early part of its germination, and that if the spores of a fungus are subjected for twenty minutes to dilute solutions of copper sulphate they will not germinate.

Wolff,³ in 1874, repeated the experiments of Kühn and confirmed

¹ Landw. Vers. Stat., 15, 1872, p. 274.

² Ibid.

³ Der Brand des Getreide, etc., Halle, 1874, p. 18.

his results. He went still further and claimed that the smuts of oats, wheat, and barley gain entrance into their hosts through the first light-colored, yellowish-green sheathing leaf.

Brefeld¹ asserts that after the green leaves have pushed their way through the primary leaf to a distance of 1 cm. or more, all danger of infection capable of causing smutted heads of grain is passed. More recent observations show that corn is an exception to this rule.

H. Marshall Ward² thinks it probable that the sporidia can gain entrance through any of the embryonic tissues and infect the host plant, developing with its fruiting organs. If, then, the fungus can enter only at the collar of the plant, a few hours in germination may make quite a difference in the future seedling.

SUMMARY OF EXPERIMENTS WITH COPPER SULPHATE TO PREVENT SMUT OF OATS, WHEAT, BARLEY, ETC.

As has been mentioned, several methods of treatment have been discovered that are more or less effective in preventing the smut of oats, wheat, barley, etc. In the case of oat smut (*Ustilago avenæ* (Pers.) Jensen), as well as allied species on other hosts, the most successful means of prevention is that known as the hot-water treatment, discovered by J. L. Jensen,³ of Denmark. It consists in treating the seed oats for fifteen minutes with water heated to a temperature of 132° to 135° F. This treatment kills all the smut spores without in any way injuring the vitality of the seed.

As has long been known, the presence of some of the salts of copper, even in dilute solutions, will prevent the germination of the spores of fungi, a fact to which the efficiency of some of our most important fungicides is due. Of the salts of copper, perhaps the most effective is the sulphate, and so extensively is this employed that its use is now almost universal. Although some writers claim that it is more injurious than beneficial, yet, when considered from an economic standpoint, the ease with which it can be applied is no doubt greatly in its favor.

Tessier⁴ seems to have been the first to use copper compounds for the prevention of smut, his experiments having been carried on in 1789. It would appear, however, that no immediate advantage resulted from his work.

Prevost,⁵ in 1807, discovered that smut spores would not germinate in the presence of soluble copper compounds.

Sinclair⁶ claims that the use of copper for the prevention of grain smuts had long been practiced throughout Flanders.

¹ Nachr. aus dem Kl. Landw., Berlin, No. 221, p. 1591.

² Gard. Chron., 5, ser. 3, 1889, p. 234.

³ Gard. Chron., 3, ser. 3, 1888, p. 555.

⁴ Art. Carie, p. 721.

⁵ Mem. Carie Charbon des blés, Paris, 1807, p. 65.

⁶ Code of Agriculture, Appendix, p. 62.

Kühn,¹ who seems to have been one of the first to systematically investigate the subject of smut infection and the means for its prevention, in 1858 recommended the use of 1 pound of copper sulphate for each 7 bushels of wheat to be treated. In his experiments the fungicide was dissolved in enough water to cover the wheat to a depth of 4 or 5 inches, and in this the grain was soaked for from twelve to fourteen hours before sowing.

Dreisch² in 1873 showed that if wheat which had been soaked in dilute solutions of copper was placed in milk of lime for a few minutes the injurious effect of the copper compound on the seed would be overcome to a great degree. He recommended the use of a 0.5 per cent solution of copper sulphate as being sufficiently strong for treating the seed.

Haberlandt³ soaked wheat before planting for six, twelve, eighteen, and twenty-four hours in 0.1, 0.5, 1, and 5 per cent solutions of copper sulphate. He found that soaking the seed for six hours in a 0.1 per cent solution was sufficient to kill all smut spores. Where the stronger solutions were used the seed was injured, the time of germination being considerably prolonged.

J. Isidore Pierre⁴ tested the effect on seed of a 2 per cent solution of copper sulphate at different temperatures. When soaked for three minutes at 60° C. 54 per cent of the seed germinated, and when soaked for the same length of time at 50° C. 63 per cent germinated.

F. Kudelka⁵ compared the germination of wheat soaked for sixteen hours in a 0.5 per cent solution of copper sulphate with similar lots of seed soaked for the same time in distilled water. Seed germinated in a Nobbe tester gave 66 per cent germination for the copper-treated seed as compared with 74 per cent when soaked in water. Seed planted in the ground and covered to a depth of 1 cm. gave 65 per cent for the copper-treated and 67 per cent for the water-soaked. When covered to a depth of 3 cm. 24 per cent of the copper-treated seed germinated, and 54 per cent of the water-soaked. Kudelka also found that soaking for two hours in the solution killed all smut spores and did not materially affect the seed.

Nobbe⁶ has shown that copper sulphate has a corrosive effect on various seeds, that the injury done to germination is in proportion to the strength of the solution used and the length of time the seed is soaked, and that the principal injury is done to the root systems. He believes that the best results are obtained by immersing seed from one to two hours in comparatively weak solutions, and that the injury is

¹ *Krankheit der Kulturgewächse*, 1859, p. 85.

² *Untersuch. über die Einwirkung Kupferlösung auf Keimung des Weizen*, 1873.

³ *Landw. Centralbl.*, 22, 1874, p. 281.

⁴ *Ann. Agron.*, 1876, p. 177.

⁵ *Oesterr. Landw. Wochenbl.*, 1876, p. 1280.

⁶ *Landw. Vers. Stat.*, 15, 1872, pp. 252-275.

done mainly to immature and diseased seed. Nobbe¹ also reports that wheat, rye, barley, timothy, Swedish clover, and mustard seed soaked in 0.1, 0.5, and 1 per cent solutions were retarded in their germination when grown on filter paper, and also calls attention to the injury done to the radicle. In the case of treated timothy seed no roots were developed even after several weeks.

Sorauer,² quoting Dreisch, says that wheat soaked in copper solutions is late in germinating, that sometimes the first leaf does not unfold, and that the injury to the root is marked.

Henslow, quoted by L. H. Bailey,³ claims that copper sulphate will cause no injury to the germination of wheat.

Larbaletrier⁴ soaked wheat for six hours in a solution of copper sulphate, the strength of which is not given, and twelve days after planting the seed found 74 per cent had germinated.

A. Voelcker⁵ says that no objection can be urged against the use of copper sulphate as a preventive of wheat smut.

James Fletcher⁶ recommends as the best treatment for smutted wheat 1 pound of copper sulphate for each 4 bushels of wheat. The copper is dissolved in 5 quarts of water and the seed soaked for ten minutes, or until all the solution is absorbed.

Shutt⁷ soaked wheat for thirty-six hours in a solution of 1 pound of copper sulphate to 8 gallons of water, and found that the germination was injured. When the solution was diluted to one-third the above strength the effect was still strongly marked. The seed was allowed to dry for thirteen days before planting, and to this much of the injury was probably due.

S. A. Bedford⁸ says that wheat treated with copper sulphate at the rate of 1 pound to 5 to 10 bushels gave less smut, a larger yield, and a heavier product than untreated seed.

A. Mackay⁹ treated very badly smutted seed wheat with copper sulphate. He used 1 pound in 3 gallons of water on 5, 7, and 10 bushels, pouring the solution over the grain until all was absorbed. The harvest from the treated seed was two days earlier, from 7 to 10 bushels more per acre, and 2 to 4 pounds per bushel heavier than on the untreated plats.

E. W. Hilgard¹⁰ recommends soaking wheat in a saturated solution for three minutes. He claims that if a weaker solution is used the seed

¹ Samenkunde, p. 274.

² Pflanzenkrankheiten, Vol. II, p. 205.

³ Mich. Agr. Expt. Sta. Rept., 1887, p. 133.

⁴ L'Italia Agricola, 1887, p. 397; Just's Bot. Jahresb., 1888, p. 12.

⁵ Jour. Roy. Agr. Soc., 14, ser. 2, 1878, p. 252.

⁶ Canada Expt'l Farms, Bull. No. 3.

⁷ Canada Expt'l Farms Rept., 1890, p. 146.

⁸ Canada Expt'l Farms Rept., 1893, p. 238.

⁹ Canada Expt'l Farms Rept., 1893, p. 278.

¹⁰ U. S. Dept. Agr. Rept., 1887, p. 278.

may be left in it until germination begins without any serious injury being done.

M. A. Scovell¹ soaked wheat in a solution of 10 pounds of copper sulphate to 8 gallons of water. All smut was prevented by the treatment, but no statement is given as to the effect of the treatment on germination.

Kellerman and Swingle² experimented with numerous fungicides for the prevention of wheat smut. They found a 5 per cent solution of copper sulphate prevented all smut when the seeds were soaked for twenty-four hours. The stand was injured a little, but at harvest the yield was two or three times that secured from untreated plats. Soaking the seed in an 8 per cent solution for twenty-four hours and then immersing it for a short time in limewater increased the yield over the check plats.

J. F. Hickman³ found that all smut spores were destroyed on wheat soaked for ten minutes in a solution of 2 to 12 ounces of copper sulphate in 12 gallons of water. On plats sown with such seed there was an increased yield of both grain and straw.

W. C. Latta⁴ experimented with a solution of copper sulphate for the prevention of loose smut and bunt in wheat. The seed was soaked for ten minutes in a solution of 4 ounces of copper sulphate to 1 gallon of water. The bunt was prevented, but the smut was not. Germination was greatly retarded, and as a consequence the yield was lower than in the case of the check plats.

J. L. Jensen⁵ conducted experiments in Denmark on four fields of grain, the seed for which had been soaked in 0.5, 1, and 2 per cent solutions of copper sulphate. The crop from these fields was entirely free from smut. The germination for the three treated lots were 100, 88, and 66 per cent, respectively.

J. Buckman⁶ says that soaking seed in copper solutions will prevent the germination not only of the smut spores adhering to the seed, but also of all diseased or immature seed, and that seed so treated will produce a good crop free from smut. He claims also that perfect selected seed will by its rapid growth produce a crop of excellent quality with but little smut.

H. Marshall Ward⁷ recommends the selection of rapidly germinating varieties of seed, and not sowing until the temperature has reached 15° to 20° C., such a temperature being more conducive to the germination of the grain than it is to the germination of the spores. Copper

¹ Ky. Agr. Expt. Sta. Bull. 11, p. 14.

² Kans. Agr. Expt. Sta. Bull. No. 12.

³ Ohio Sta. Bull., Vol. IV, No. 4.

⁴ Ind. Sta. Bull. No. 41.

⁵ Om Kornsorternes Brand.

⁶ Jour. Roy. Agr. Soc., 17, ser. 1, 1856, p. 174.

⁷ Gard. Chron., 5, ser. 3, 1889, p. 268.

sulphate, he says, is a good smut preventive, but is objectionable on account of its injury to the seed.

Sorauer¹ recommends soaking seed for sixteen hours in a 0.5 per cent solution as a preventive of grain smut. When seed was soaked for twenty-four hours in a 1 per cent solution 4 per cent of it was destroyed.

W. Carruthers² says that where seed is soaked for too long a time in copper solutions the terminal cells of the radicles are destroyed. In some cases the plumules of the injured seeds protrude and are nourished for a time by the starch in the seed, but the plant fails to produce roots and finally dies.

Strebel³ tested the effect on seed of applying bordeaux mixture to the soil. He applied 900 liters of 2, 3, and 4 per cent solution per hectare, and sowed wheat, oats, and rye. The plants came up so quickly and grew so well that he thought there could have been no injurious effect from the copper sulphate as applied.

R. Otto⁴ claims that copper solutions have an injurious effect on the roots and terrestrial parts of corn, beans, and peas, causing an abnormal growth of these parts.

S. A. Beach⁵ investigated the effect on wheat, peas, and tomatoes of placing copper in the soil. The soils in which these seeds were grown contained 2 and 5 per cent, respectively, by weight of dry copper sulphate.

In the soil containing 5 per cent 12 per cent more wheat, 22 per cent more tomatoes, and 27 per cent more peas germinated than in the check, although the seed in the copper-treated soil was somewhat slower in coming up. In the soil containing 2 per cent of copper sulphate 17 per cent more peas, 6 per cent more tomatoes, and 17 per cent less wheat germinated than in the check. All plants grown in the treated soils were of a darker green color than those in the checks, the color becoming deeper in proportion to the strength of the mixture. The plants made a rather unsatisfactory root growth.

A. Bruttini⁶ states that wheat soaked for twenty-four hours in 1 and 2 per cent solutions gave 86.6 and 60 per cent germination, respectively, at the end of eighteen days.

Jensen⁷ reports that a 0.25 per cent solution practically prevented smut in oats, although the total germination of the seed was slightly lowered by its use. When oats were soaked for twelve hours in a 1 per cent solution 75 per cent failed to germinate, and the growth of those which germinated was so greatly retarded that the plants were

¹ Pflanzenkrankheiten, Vol. II, p. 205.

² Jour. Roy. Agr. Soc., 17, ser. 2, 1881, p. 289.

³ Die Weinlaube, No. 44, 1893, p. 521.

⁴ Naturw. Wochenschr., 1893, p. 565.

⁵ N. Y. State Sta. Bull. No. 41, n. ser.

⁶ Staz. Sper. Agr. Ital., No. 27, 1894, p. 30.

⁷ Jour. Roy. Agr. Soc., 24, ser. 2, 1888, p. 409.

still green when the untreated plats were ready for harvesting. When the seed was limed after having soaked for twelve hours much of the injury was prevented.

J. C. Arthur¹ recommended that seed oats be soaked for twenty-four hours in a solution of 4 ounces of copper sulphate to each gallon of water. If the strength of the solution be increased four times, two hours' soaking will be sufficient. The same author² soaked oats for five minutes in a solution of 1 pound of copper sulphate to 1 gallon of water. The smut was greatly reduced by the treatment, but the germination was two days later and the yield 4 bushels less per acre than in the adjacent plats of untreated seed. A lot of seed treated in the same way gave in a tester 67 per cent germination as compared with 98 per cent for a similar lot of untreated seed. In the case of the treated seed the germination was abnormal, sometimes the radicle protruding first and at other times the plumule, and in many cases when the roots did appear they sprang from the first or second nodes of the young stem. In another instance Arthur placed in a Geneva tester a lot of 42 treated seeds which had lain two days in the ground. At the end of seven days there were 41 plumules, 34 of which appeared the first two days. The roots began to push out the second day, 11 appearing on the third day and the last on the fourteenth day. In another experiment he found that germination was greatly delayed and subsequent growth checked by the use of copper sulphate, although there was no question of the treatment preventing the smut.

C. S. Plumb³ found that soaking oats for seventeen and a half hours in a solution of 4 ounces of copper sulphate per gallon gave a crop containing 2 per cent of smutted heads, while soaking for forty hours entirely destroyed the smut spores.

Kellerman and Swingle⁴ experimented in 1890 with fungicides for the prevention of oat smut. They found that seed soaked in a 0.5 per cent solution of copper sulphate for twenty-four hours gave at harvest an increased yield, entirely free from smut. Stronger solutions injured germination, and a strength of 9.9 per cent killed all seed.

W. T. Swingle⁵ recommends soaking oats for twelve hours in a solution of 1 pound of copper sulphate to 24 gallons of water, and immersing the seed in limewater for from five to ten minutes before planting.

Hollrung⁶ treated barley for sixteen hours with a 0.5 per cent solution of copper sulphate, and afterwards soaked it for five minutes in limewater. The treated seed gave 89 per cent germination and the check lot 98 per cent. The amount of smut was as 1 to 30 for the

¹ Ind. Sta. Bull. No. 28.

² Ind. Sta. Bull. No. 35.

³ N. Y. State Rept., 1886, p. 124.

⁴ Kans. Sta. Bull. No. 15, p. 109.

⁵ U. S. Dept. Agr. Farmers' Bull. No. 5.

⁶ Braunsch. Landw. Ztg., 52, 1894, No. 51, p. 214.

treated and untreated lots, and the yield of grain and straw was increased by the treatment.

Hermanauz¹ experimented with copper sulphate on barley. He found that germination was retarded and to some degree destroyed by the treatment, and claims that all the plant's organs are greatly weakened by the corrosive action of the fungicide. In the treated plants the leaves roll up badly and the root system is poorly developed. There is little or no starch present anywhere in the roots, and frequently an absence of root tips. He also claims that it is the sensitive growing tip of the radicle that is injured and not the dormant embryo. The effect of copper treatment may be neutralized, he says, by planting the seed in the ground, by treating it with lime, or by thoroughly washing it after soaking in the fungicide. The corrosive action of the copper sulphate, he states, is entirely upon the embryo and not upon the endosperm.

A. A. Crozier² tested the germination of corn and wheat when soaked in a 0.5 per cent solution of copper sulphate, with the following results:

	Soaked 10 minutes.	Check.	Soaked 5 hours.	Check.	Soaked 24 hours.	Check.
Corn.....	92	95	91	93	93	93
Wheat.....	80	85	48	85	37	93

C. E. Bessey³ for the prevention of smut in corn recommends soaking the seed for fifteen to twenty minutes in a solution of 1 pound per gallon, and to increase or diminish the time of soaking as the strength of the solution is increased or diminished.

A. W. Pearson⁴ soaked corn in strong bordeaux mixture for four hours before planting. The germination was to a great degree prevented.

L. R. Jones⁵ experimented with corn soaked in 0.5, 1, and 6.25 per cent solutions for twenty-four hours; in 6.25 per cent solution for ten hours, and in 6 and 12 per cent solutions for five, ten, fifteen, and sixty minutes. Only in the seed soaked for twenty-four hours was the germination materially injured.

L. H. Pammel⁶ conducted experiments in the soil with several mixtures containing copper salts, and claims that corn grown in such soils was injuriously affected in its root systems; in some cases the totals of germination were considerably reduced.

Pammel and Stewart⁷ conducted some experiments with bordeaux

¹ Keimung des Gerstenkornes, Darmstadt, 1876.

² Jour. Mycol., Vol. VI, p. 9.

³ Nebr. Sta. Bull., Vol. I, No. 11, p. 34.

⁴ Garden and Forest, 4, 1891, p. 498.

⁵ Vt. Sta. Rept., 1891, p. 138.

⁶ Iowa Sta. Bull. p. 321, No. 16.

⁷ Agr. Sci., 8, 1894, No. 5, p. 215.

mixture, eau celeste, and ammoniacal copper carbonate on oats and corn. The experiments seemed to show that all but bordeaux mixture reduced the germination.

Hitchcock and Carleton¹ report the effect of copper sulphate on corn. Solutions of 5 and 10 per cent strength were used, the seed being soaked for twenty-four, forty-eight, and seventy-two hours. It was found that seed soaked in the 5 per cent solution for forty-eight hours or in the 10 per cent solution for seventy-two hours gave less than 50 per cent germination, while that soaked in the 10 per cent solution for twenty-four and forty-eight hours gave between 50 and 80 per cent germination. The effect was practically the same for wheat, lettuce, mustard, alfalfa, tomato, and castor bean seed.

Brefeld,² in recent experiments, showed that corn smut can not be prevented by treatment of the seed, as the fungus can gain access to any meristematic tissue of the host and set up a local infection.

In India³ sorghum seed is commonly treated with 0.5 ounce of copper sulphate dissolved in water enough to cover the seed required for 1 acre. Where such treatment is followed no smut is found.

At the Mississippi Station⁴ five tomato seeds each were planted in thirty boxes containing soil with 0.5 per cent of dry copper sulphate. The germination ranged from 0 to 52 per cent. The plants produced were weak and of a bluish color. The same boxes were seeded again, the seed in this case germinating well; however, the plants produced were weak and all soon died.

G. McCarthy⁵ claims that 1 gram of copper sulphate per liter of water will retard the germination of most seeds, especially those of leguminous plants, and that in most cases the germinative ability of one-third or more will be destroyed.

Haselhoff⁶ investigated the effect of copper on plants, and found that soluble salts are injurious to them when applied in solutions containing 10 mg. of copper oxide per liter. In smaller quantity very little if any injury was done. He claims that copper salts are more injurious to oats and barley seed than to grass seed, and more so to corn than to beans. Wherever there was an abundance of lime in the soil the injurious action was to a considerable degree prevented.

Vedrodi⁷ found that garden soils in his region contained an average of 0.06 to 0.08 per cent of copper oxide, and no effect could be noticed either on germination or on the subsequent growth of anything that was planted there, although on analysis the proportion of copper found

¹ Kans. Sta. Bull. No. 41.

² Untersuchungen aus dem Gesamtgebiet der Mykologie, 1895, No. 11.

³ Khandesh Expt'l Farm Rept. 1892, Mar. 31.

⁴ Miss. Sta. Rept., 1893, p. 56.

⁵ N. C. Sta. Bull. No. 108, p. 370.

⁶ Landw. Jahrb., 21, 1892, No. 1 and 2, p. 263.

⁷ Chem. Centralbl., 1, 1894, p. 432.

in some of the plants was seen to be four times greater than that in the soil.

A. N. Berlese and L. Sostegni¹ have shown that calcareous soils will absorb large quantities of sulphate of copper without causing serious injury to plants growing therein.

Vermorel² applied copper sulphate in the form of bordeaux mixture to soil at the rate of about 30 pounds per acre without effect. He then applied to several plats amounts that would represent the accumulation at this rate of 50, 100, 200, 300, and 1,000 years, and seeded all with wheat. Taking 10 as the yield for plats receiving no treatment, the others would be expressed by 9, 7, 5, 3, and 2, respectively, showing that the copper in the soil has comparatively little effect on the plants.

The foregoing abstracts present many contradictory opinions relative to the use of copper sulphate for the prevention of smut, and the writer conducted the following investigations with the hope that something might be developed which would harmonize the diverse opinions or add some facts relative to the nature and amount of injury done to seeds by the use of copper solutions as fungicides.

EFFECT OF COPPER SULPHATE AS SHOWN BY THE GERMINATION OF SEED.

Since oats are very liable to attacks of smut, which causes annually an estimated loss of about 8 per cent of the entire crop,³ experiments for its prevention were conducted with this grain, the seed of Scotch White Superior variety from the crop of 1894 being used.

The plan of the experiment was to treat equal quantities of seed in solutions of known varying strengths for different periods of time, after which the seed were tested for their germinative ability. Duplicate and check lots were tested, so that the figures represent averages and not isolated experiments. Lots of 100 seeds each were counted and placed in solutions of copper sulphate of the following strengths: 0.5, 1, 2, 3, 5, and 10 per cent. Each series was allowed to remain in solutions fifteen and thirty minutes, and one, two, and three hours, the experiment requiring 700 seeds for each series. After having been allowed to soak for the desired length of time the seeds were removed from the solution, dried, and planted in a bed in a forcing house, being covered 0.5 inch with the sand. The experiments were begun January 24 and were terminated April 29. During this period the temperature of the house varied from a minimum of about 50° F. to a maximum of about 90°. At the same time that the lot of seed was put in the solution of copper there was a similar lot from the same parcel placed in water, both remaining immersed for the same length of time. The

¹ Staz. Sper. Agr. Ital., 21, 1891, p. 229.

² Rev. Sta. Viticole, Villefranche, 1890, No. 3, p. 187.

³ W. T. Swingle, U. S. Dept. Agr. Yearbook, 1894, p. 413.

seed treated with water was sown beside that treated with copper, the former being used as a check lot.

A seed was considered as having germinated when its plumule appeared above the sand of the bed. All such were counted every day until the tenth day after treatment, and again on the fifteenth day, when the germination test was considered at an end. All the records were reduced to percentages and tabulated, and in this way the effect of the treatment on germination could be readily seen. Germination began the third day after planting, hence the record in the table begins from the third day. The presence of the chemical was apparent in the color of all seed soaked for thirty minutes or more in strengths exceeding 1 per cent, as well as in solutions of less strength when treated for more than one hour.

In order to test the amount of copper deposited on the oats, seeds were treated for one hour with a 1 per cent solution, after which they were thoroughly air-dried. They were then placed in distilled water, where they were allowed to remain for fifteen minutes, after which the solution was tested for copper. With ammonia a perceptible bluish color was noticed, while the potassium ferrocyanide test showed quite a distinct discoloration.

The amount of moisture taken up by oats during the process of imbibition was ascertained on account of the practical bearing it has on the use of the fungicide where large quantities of seed are to be treated. One ounce of seed was soaked for one hour in a 1 per cent solution of copper sulphate. During that time the seed took up 16 c. c. of the solution, and at the end of twenty-four hours it had taken up 24 c. c.; in other words, 66.7 per cent of the moisture taken up by the oats was absorbed during the first hour. At this rate 8.67 quarts of liquid would be taken up by each bushel of oats during the first hour of treatment. The solution remaining after the oats had been soaked was found to be stronger than it was at the beginning of the experiment, proportionally more of the water having been taken up than of the copper sulphate, indicating that the same solution can not be used over and over again where it is desired to treat seed with a definite strength of the solution.

Turning now to the detailed statement of the percentage of germinations secured for the respective methods of treatment, the following table gives the data obtained:

Germination of oats treated with copper sulphate solutions for different periods.

	Third day.	Fourth day.	Fifth day.	Sixth day.	Seventh day.	Eighth day.	Ninth day.	Tenth day.	Fifteenth day.
Seed soaked for 15 minutes:	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>
Check (untreated).....	9	79	95	97	98	98	98	99	99
In 0.5 per cent solution.....	9	72	93	93	99	99	99	99	100
1 per cent solution.....	0	68	93	98	99	99	99	99	99
2 per cent solution.....	3	40	63	79	83	86	86	86	86
3 per cent solution.....	3	43	60	85	89	89	89	89	83
5 per cent solution.....	7	45	67	82	82	82	82	82	84
10 per cent solution.....	0	9	16	26	36	48	56	65	65
Seed soaked for 30 minutes:									
Check (untreated).....	16	84	97	98	99	99	99	99	99
In 0.5 per cent.....	3	50	77	88	95	95	95	96	97
1 per cent solution.....	5	47	69	87	89	92	93	94	94
2 per cent solution.....	0	8	13	31	36	50	58	64	72
3 per cent solution.....	0	5	22	29	31	32	32	32	39
5 per cent solution.....	0	5	10	14	21	21	21	21	26
10 per cent solution.....	0	0	2	5	6	9	15	19	19
Seed soaked for 1 hour:									
Check (untreated).....	16	84	97	98	99	99	99	99	99
In 0.5 per cent solution.....	8	57	79	85	88	91	91	93	94
1 per cent solution.....	0	17	45	60	77	85	85	86	88
2 per cent solution.....	0	2	9	18	30	42	47	53	54
3 per cent solution.....	0	1	5	15	24	32	34	37	37
5 per cent solution.....	0	4	7	9	14	15	16	17	17
10 per cent solution.....	0	2	4	8	10	11	16	18	18
Seed soaked for 2 hours:									
Check (untreated).....	8	64	96	99	99	99	99	99	99
In 0.5 per cent solution.....	2	27	56	69	81	89	94	96	96
1 per cent solution.....	0	9	25	37	56	66	81	81	93
2 per cent solution.....	0	0	0	4	6	14	33	40	46
3 per cent solution.....	0	0	0	2	4	11	16	21	22
5 per cent solution.....	0	0	0	0	1	4	4	6	7
10 per cent solution.....	0	0	0	0	1	1	2	2	3
Seed soaked for 3 hours:									
Check (untreated).....	7	70	94	99	99	100	100	100	100
In 0.5 per cent solution.....	2	20	54	73	81	91	97	98	99
1 per cent solution.....	0	3	8	23	37	59	79	83	89
2 per cent solution.....	0	0	0	3	8	13	27	31	41
3 per cent solution.....	0	0	0	0	3	7	16	18	23
5 per cent solution.....	0	0	0	0	1	1	2	3	4
10 per cent solution.....	0	0	0	0	2	2	4	4	4

From the foregoing table it will be seen that the germinative ability of the untreated seed was in excess of 99 per cent, and this figure is closely followed by the seed treated with 0.5 per cent and 1 per cent solutions for the various times of treatment. In the case of the seed soaked for fifteen minutes the germination of the check lot was surpassed by the lot treated with the 0.5 per cent solution and equaled by that soaked in the 1 per cent solution. For the other times of treatment there was a slight falling off, as shown in the table. For the solutions stronger than 1 per cent the effect upon germination is shown in the rapidly decreasing percentages as the strengths of the solution or time of soaking is increased.

The extent to which the treatment retards or prevents germination is also shown in the table. In the case of the check lots of seed practically all germination was effected by the sixth day; on the other hand,

for the weaker solutions, with the exception of the seeds treated for fifteen minutes, it was only after the seventh day that a considerable portion germinated. Germination continued until the ninth and tenth days, and in some cases until the fifteenth day, when the test was terminated. In the case of the stronger solutions the delay in germinating is still more apparent, in some cases not a seed sprouting before the sixth day after the treatment, at which time nearly all the check plants were up.

The character of the plants grown from seed treated with the weaker solutions was not perceptibly different from that of plants from seed which received no treatment. When, however, the stronger solutions were used some very marked effects were noticeable, the most evident of which, aside from destroying or greatly retarding germination, was in the root development.

EFFECT OF COPPER SULPHATE ON THE ROOT SYSTEM OF PLANTS.

The corrosive effect of copper sulphate upon the root system of seedlings has already been noticed by Nobbe, Arthur, and others (pp. 9, 13). Some of the plants grown from seed treated for three hours in a 2 per cent solution and most of those from the 5 and 10 per cent solutions were entirely without primary roots at the end of the experiments. Unsprouted seed after lying for eight days in the sand was examined and the radicle end of the embryo was found to be dead and black.

In many of the sprouted seeds examined the slightly developed primary root was eroded or so softened as to break down easily under the slightest pressure. Plants whose plumules had grown to 10 to 15 cm. in height had no roots other than the rotted remnants of the primary roots. All such plants withered and died as soon as the reserve material of the seed was exhausted. In other cases secondary roots by which the plant could be sustained were developed from the first few nodes, often appearing as far as an inch from the seed. In nearly every case examined the roots developed from the primary or embryonal node were unable to push their way through the glumes of the seed, and remained as more or less disintegrated remnants. However, after two or three weeks but little difference in general appearance could be seen between plants developing secondary roots and those grown from seed which had received no treatment.

EFFECT OF COPPER SULPHATE ON THE GROWTH OF AERIAL PARTS OF PLANTS.

In the aerial portion of the plants the effect of the stronger solutions was apparent in the retarded growth and in the difference of color. At the expiration of ten days no difference was noticeable between the plants from the untreated and treated seed in cases where only the weaker solutions had been used, but in plants from seed which had

been treated with the stronger solutions the leaves rolled up badly, in some cases never expanding. It frequently happened that the primary sheathing leaf remained unsplit, in which case the later leaves, prevented for a time from pushing out, doubled themselves into fantastic shapes, and finally split the sheath leaf on the side, pushing out partly or wholly through the lateral opening forced by the bend in the growing stem.

Two lots of plants, the seed for which had been soaked for two hours, were allowed to grow for thirty days, when all were carefully taken up and measured, with the results given in the following table:

Height of two lots of plants one month after planting, seed having been soaked for two hours.

Strength of solution.	Under 5 cm.	5-10 cm.	10-15 cm.	15-20 cm.	20-25 cm.	25-30 cm.	30-35 cm.	Total plants.
No solution (check)....	2	11	7	10	70	64	28	192
0.5 per cent.....	2	6	10	32	41	63	38	192
1 per cent.....	8	10	17	21	49	44	24	173
2 per cent.....	15	15	12	17	27	7	4	97
3 per cent.....	13	15	12	16	8	2	0	66
5 per cent.....	11	7	4	8	1	0	0	31
10 per cent.....	8	7	7	2	0	0	0	24
Total	59	71	69	106	196	180	94	775

The above figures express the height of all plants grown from 1,400 seeds planted at two different times and allowed to grow for thirty days in the sand of the forcing bed. In the first column are included all those seeds which had germinated, but failed, even after so long a time, to appear above ground. The height was measured from the surface of the ground to the extremity of the longest leaf. In the totals it will be seen that the check lots and those treated with the 0.5 per cent solution gave the same number of plants, and the average height of both was about the same. At the time of measuring the plants but little if any difference could be noticed in their aerial parts. The third series, those treated with a 1 per cent solution, were nearly equal in height to the lots untreated and treated with 0.5 per cent solution, and to all appearance were well developed. All other plants were conspicuously affected by the treatment. One of the most striking differences noticed between the plants from the treated and the untreated seed was in color, those from the treated seed being a deep bluish-green, while those from seeds soaked in solutions of 3 per cent or more for over thirty minutes were of a much darker green. This difference was in some cases maintained during the entire period of growth of the plants.

Equal weights of the aerial portions of plants, representing the most marked differences in color, were placed in alcohol and kept for a considerable time. The alcoholic solutions of the chlorophyll showed as great differences as were seen in the plants. Whether the copper acts

as a chemical stimulus in producing a greater number of chlorophyll grains by external effect, as claimed by Rumm,¹ or whether it is due to the probable combination of the copper with the cyanophyll, as claimed by Berlese and Sostegni,² causing a greater proportion of the latter as compared with the xanthophyll, it is not intended to state; however, as the last-named authors have shown that a small quantity of copper may be absorbed by plants and find its way to the aerial parts without injuring them, their explanation seems more reasonable than that of the external chemical stimulus theory.

PARTS OF SEED AFFECTED BY COPPER SULPHATE.

An experiment was undertaken which was designed to show what part of the seed is injured by copper sulphate during the process of imbibition. Four lots of seed were selected so as to be as nearly uniform in quality as possible, the glumes being carefully removed. The seeds of two lots were then cut so as to remove about one-third of the grain from the end opposite the embryo, leaving the starch well exposed. Half was soaked in water and half in a 2 per cent solution of copper sulphate for thirty minutes. The seed of the other two lots were slightly punctured with a fine needle, so that the epidermis over the embryo was pierced, after which they were soaked as above, and the four lots planted as in the other experiments. One week after planting the seed had germinated as follows:

	Per cent.
Lot 1, end cut off and soaked in water.....	90
Lot 2, end cut off and soaked in 2 per cent solution	44
Lot 3, seed punctured and soaked in water.....	40
Lot 4, seed punctured and soaked in 2 per cent solution.....	0

The results show that the effect is exerted almost wholly upon the embryo. From the low germination of lot 3 it is probable that some mechanical injury was done the seed whereby its vitality was impaired. If lots 1 and 2 are compared with the similar lot on page 18 it will be seen that they show nearly the same germinations, so that but little injury could have been done the seed by the removal of the glumes.

It has been suggested by R. Otto³ that the injury done to the embryo is caused by the corrosive effect of the copper sulphate acting upon it while the protoplasm is yet dormant, and that the living protoplasm will prevent the osmotic entrance of the copper to the cells, thus preventing their destruction. This presupposes the inability of copper to be absorbed and carried into the aerial parts of plants, a theory denied by many authors and shown to be fallacious by some of the foregoing experiments.

¹ Ber. Deut. Bot. Ges., Vol. X, No. 2, p. 79.

² Rev. Internat. Vit. et Enol., 1, 1894, No. 11 and 12, p. 399.

³ Naturw. Wochenschr., 1893, p. 565.

PREVIOUS SOAKING OF THE SEED IN WATER A MEANS OF REDUCING
THE INJURY FROM COPPER SULPHATE.

On March 22 three lots of seed were placed between layers of moist blotting paper and kept under a bell jar for forty-eight hours, or until germination had well begun, as was shown by the protrusion of the radicles to a distance of 1 or 2 mm. In this condition the germinating seeds were treated for thirty minutes with 3, 5, and 10 per cent solutions of copper sulphate, after which, without drying, the seeds were planted in sand and covered as before. The object of this experiment was to test the claim of Otto that "the active protoplasm of the cell is able to resist the osmotic entrance of copper." The plumules began to appear the second day after planting, or the fourth day from the beginning of the experiment. The detailed record of the germinations are as follows:

Daily germination of oats treated after sprouting had begun.

Strength of solution.	Fourth day.	Fifth day.	Sixth day.	Seventh day.	Eighth day.	Ninth day.	Tenth day.	Fifteenth day.
3 per cent.....	6	49	68	75	76	81	84	84
5 per cent.....	4	16	45	52	57	60	64	64
10 per cent.....	0	2	32	40	43	44	45	48

By comparing the total number of germinations with the lots of seed receiving the same treatment without the preliminary germination, as is shown on page 18, under the thirty minutes' treatment, it will be seen that in the lot of dry seed treated with a 3 per cent solution 39 per cent germinated, and in the lot where the seeds began to sprout before treatment 84 per cent germinated, or a gain for the latter lot of 115 per cent. For those soaked in the 5 per cent solution the figures for the respective lots were 26 and 64 per cent, a gain in this case of 133 per cent. Comparing the lots soaked in the 10 per cent solution, the germinations were 19 and 48 per cent, a gain of 148 per cent in favor of the seeds whose germination had begun before their treatment with the fungicide.

Judging from the greatly increased percentage of germination shown by the lots of seed that were sprouted before soaking in the copper solution, it would seem that the active protoplasm in the embryo is able to withstand the corrosive action of the stronger solutions of copper sulphate to a much greater degree than is possible for that in the dormant embryo. However, when the root systems and aerial portions of the plants were examined it was seen that but little, if any, advantage could have accrued to the plant from using the fungicide at the later time. In only two or three cases could it be said that there had been any development of primary roots, and in many instances the plants died before lateral roots were put out for their support. The aerial portions of the plants showed the same dark bluish-green color

and tightly rolled leaves already mentioned as possessed by plants from seed treated with the stronger solutions of copper sulphate.

In these experiments it was desired to learn the effect of copper sulphate alone, hence it was the only form used. Tschirch¹ has shown by cultures that copper is injurious to plants only when applied in the form of the soluble salts, such as the sulphate, nitrate, chloride, etc. In experiments made with 36 kinds of plants in water cultures, where the nutrient solution contained copper oxide (an insoluble compound) in considerable quantity, stronger and better appearing plants were produced than those grown under similar conditions, except that there was no copper in their solutions.

It is a well-known fact that carbonate of lime unites with copper sulphate, forming a nearly insoluble compound. In order that there should be no diminution of the effect of the copper sulphate, all seeds were planted in almost pure sand, which could not have contained much if any lime. It was intended at one time to conduct a series of experiments to test the ameliorating effect of lime when applied to the seed and also when present in the soil, but this was not found necessary, as all experimenters agree that either milk of lime or powdered air-slacked lime applied to seed which have been soaked for many hours in a solution of copper sulphate, or that lime in the soil capable of forming a carbonate, will greatly overcome the injurious action of the soluble salts of copper. Lime in the soil is immediately attacked by the copper on the treated seed and an insoluble compound formed, and so long as an excess of lime is present the corrosive effect of the copper sulphate on the seed is destroyed. It is highly probable that many persons who have unqualifiedly advocated the use of strong solutions of copper sulphate for the prevention of smut owed their successful use of such strong solutions to the presence of a considerable portion of lime in the soil where the seed was planted. The action of lime upon the small quantity of copper at once renders it insoluble and no further injury can be done the seed, while the smut spores have already become powerless to germinate and infect the plant.

SUMMARY.

(1) Copper sulphate, as has been shown by many investigators and by its long and widely extended use, is capable of preventing to a greater or less degree the attacks of grain smuts when the seed is soaked in solutions of it prior to sowing.

(2) Copper sulphate in solution destroys or retards germination of seed soaked in it. The amount of injury done depends upon the strength of solution and length of time the seeds are soaked. The injury is due to the corrosive effect of the fungicide on the embryo, which causes the root systems to be greatly stunted. The stronger

¹ Das Kupfer vom Standpunkte der Chemie, Toxicologie, und Hygiene, 1893.

solutions destroy nearly all the primary roots and stunt the plant so as to often prevent the development of lateral roots in time to sustain the young plant. It also causes an abnormal growth of the aerial parts of the plant, often resulting in distorted stems and tightly rolled leaves.

(3) Solutions of 0.5 per cent and 1 per cent copper sulphate may be used for treating oats without doing serious injury to the seed. Numerous investigators quoted in the earlier portion of this paper have shown that by the use of solutions of such strengths for one to two hours it is possible to prevent most, if not all, smut infection, and if confidence is to be placed in their experiments there is no necessity for using solutions of greater strength or for longer time.

(4) When stronger solutions are used or the period of soaking is greatly extended, the seed should receive a second soaking for from five to fifteen minutes in limewater, or should be dusted over with powdered air-slacked lime to prevent the long-continued action of the fungicide.

(5) It seems probable that soaking seeds in water for a few hours prior to subjecting them to the copper solution might prove beneficial, as it is shown in this paper that such treatment greatly increased the germination of seed soaked in the stronger solutions. No experiments are known to have been made to ascertain the effect of such treatment on smut prevention, and such treatment might not prove advantageous when tested on a large scale.

(6) Much stronger solutions of copper sulphate may be used without injury, provided the seed after soaking are immediately placed in the soil instead of being tested in germinators or grown in water cultures or in sand. This is probably due to the presence of lime in the soil forming an insoluble oxide of copper, as well as the leaching away of some of the adherent copper from the seed.

(7) Copper in an insoluble form is probably not injurious to plants, and it is only the corrosion induced by the action of the soluble sulphate that causes the injury to the seed.

